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Yield and Persistence of Tall Fescue in the Southeastern Coastal Plain after Removal of Its Endophyte

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ABSTRACT

The coastal plain of the southeastern USA lacks a dependable perennial cool season forage crop, but tall fescue (*Festuca arundinacea* Schreb.) germplasms have been developed which show greater persistence and yield than currently marketed cultivars in this region. Since these germplasms were found to be infected with the tall fescue endophyte (*Acremonium coenophialum* Morgan-Jones and Gams), the contribution of the endophyte to their performance was unknown. The objective of this study was to compare yield and stand survival of endophyte-infected (EI) and endophyte-free (EF) (achieved by removal of its endopbyte) versions of four persistent germplasms (GA-5, GA-Jesup, GA-Jesup Improved, and GA-Ecotype KY31) in clipped small plots at three locations in Georgia (Americus and Tiflon in the coastal plain and Watkinsville located in the fescue growing area of the southern piedmont region) for a 3-yr period. The EI version of each germplasm showed greater stand survival and yield than its EF version at Americus and Tifton, but no differences were recorded for infection status for the same parameters at Watkinsville. These findings indicate that endophyte removal greatly reduces the ecological fitness of tall fescue by possibly allowing less tolerance to summer drought. Presently, only EI tall fescue can be dependably recommended for perennial pasture in the southeastern coastal plain.

ALTHOUGH TALL FESCUE is one of the most widely used pasture grasses in the USA, its use in many areas of the couhtry is restricted because of poor persistence. One such area is the coastal plain region of the southeastern USA where heat and drought stress, competition from aggressive warm season grasses, nematodes, disease, insect pests, and poor, acidic soils all serve to eliminate tall fescue stands. However, overwintering cattle in this region is both difficult and expensive because of the lack of a dependable, perennial cool season forage crop.

A breeding effort in Georgia to increase persistence in tall fescue resulted in germplasms which showed better persistence than currently marketed cultivars in the coastal plain region during the mid-1980s (Bouton, 1986). About this time, studies were being published that demonstrated that infection with Balansiae fungi enhanced survival and growth rates of some grasses (Clay, 1984). In the case of tall fescue in north central Texas, both stand maintenance and forage availability were improved in pastures showing high levels of infection with endophytic fungi (A. *coenophialum*) compared to pastures with low infection levels (Read and Camp, 1986). Since the Georgia germplasms were found to be highly infected with endophytic fungi (J.H. Bouton, 1987, unpublished data), the contribution of the endophyte to their persistence and performance was unknown. Therefore, this study was undertaken to investigate the effects of endophyte removal on the growth and stand survival of persistent tall fescue germplasms in the southeastern coastal plain.

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MATERIALS AND METHODS

Four EI tall fescue germplasms, GA-5, GA-JES, GA-JESIMP, and GA-ECOKY31, were used in these studies. These germplasms are currently experimental and have not been officially released. The EF versions of GA-5, GA-JES, and GA-ECOKY31 were produced by long term seed storage of the EI seed (>3 yr). Long-term storage is reported to kill the endophyte with little damage to the seed embryo (Bacon and De Battista, 1991). The stored seed and freshly harvested seed from the EI version of each of these germplasms were planted and seed increased one more generation. The increased seed was then used in this current study. In the case of GA-JESIMP, the following procedure was used to quickly remove the endophyte: Seed was placed in a sulfuric acid dessicator and then put into an oven at 47 °C for 7 d. The relative humidity within the dessicator was 45% which was achieved by placing in the bottom a mixture (vol/vol) of 75% glycerol and 25% water. The seed were suspended above this mixture by a wire frame. This EF GA-JESIMP seed and the original EI GA-JESIMP were not increased, but used directly in this study.

Prior to planting, seed of each germplasm were germinated and 54 randomly selected seedlings grown in the greenhouse. Preplant infection levels for each germplasm were determined from 8-wk-old seedlings. The presence of the endophytic hyphae was assessed in stem pith samples stained with aniline blue by procedures described by Bacon et al. (1977). For the EI germplasms, the preplant infection levels were as follows: GA-5 = 85%, GA-JES = 98%, GA-JESIMP = 96%, and GA-ECOKY31 = 98%. All EF germplasms were 0% infected.

The eight entries (four germplasms x two infection levels) plus the EF cultivar AU-Triumph were planted in plots in a randomized complete block design with six replications at three locations (Americus, Tifton, and Watkinsville, GA). Watkinsville is in the southern piedmont region of Georgia, a prominent tall fescue growing area, and was considered the control area representing an environment normal for good fescue growth and survival. Tifton and Americus are in the upper coastal plain region and are considered outside the area of adaptation

Abbreviations: EF, endophyte-free; EI, endophyte-infected; GA-JES, GA-Jesup, GA-JESIMP, GA-Jesup Improved; GA-ECOKY31, GA-Ecotype KY31.

Table 1 Seasonal dry matter production and stands (visual estimation of percent of ground covered with tall fescue) of endophyte-infected (EI) and endophyte-free (EF) versions of four tall fescue germplasms (data pooled for germplasms) when tested at Americus, GA during 1988.

Status	Yield			Stands	
	Winter	Spring	Fall	May	Nov
	kg ha^{-1}			%	
EI	378*	5792**	3510*	75*	91**
EF	260	4601	0	61	0

*,** Significant F test at the 0.05 and 0.01 level, respectively.

Table 2. Seasonal dry matter production and stands (visual estimation of percent of ground covered with tall fescue) of endophyte-infected (EI) and endophyte-free (EF) versions of four tall fescue germplasms (data pooled for germplasms) when tested at Watkinsville, GA for 3 yr (1988-1990).

status	Yield			Stands	
	Winter	Spring	Fall	May	Nov
	kg ha^{-1}			%	
EI	883	3320	3128	96	98
EF	793	3339	3162	94	96

of tall fescue. All tests were planted in October, 1987. Each plot measured 1.5 by 4.5 m. The soils at Americus was an Orangeburg sandy loam (fine, loamy, siliceous, thermic Typic Kandiudults), the soil at Tifton was Clarendon loamy sand (fine-loamy, siliceous, thermic Plinthaquic Paleudults), and the soil at Watkinsville was Cecil sandy loam (clayey, kaolinitic, thermic, Typic Kanhapudults). The experimental areas were initially limed with dolomitic limestone to achieve a starting pH of 6.0. Nitrogen, P, and K were then applied at 100, 75, and 75 kg ha^{-1}, respectively, and reapplied each year in September at an equivalent rate.

Forage dry matter yield was determined for each plot with a flail (Americus and Watkinsville) or sickle-bar (Tifton) mower and dry matter concentration determined with a subsample, which was oven dried. At each location, yield assessments began in February, 1988 and were repeated for 3 yr to give the following production determinations: Fall (September-December; 2 harvests), Winter (January-February; 1 harvest), and Spring (March-June; 3 harvests). In September of each year, all experimental areas were mowed and refertilized and accumulated growth was discarded. During February, March, May, and November of 1988 only, tiller number per square meter was recorded by counting all tillers in three randomly placed 0.093 m^{-2} quadrats in each plot. During May (spring) and November (fall) of each year, a visual estimate of stand (percentage of ground area covered with fescue) was made on each plot, and 10 tillers were randomly removed and assessed for the presence of the endophyte by the staining procedures described by Bacon et al. (1977).

All data were analyzed by analysis of variance (AOV) and means separated by LSD. Endophyte infection data were analyzed separately by infection status and were converted to percentage infection per plot at each sampling date. Percent infection data were then subjected to an arcsin transformation before analysis for the EI germplasms, but due to the presence of a large number of zeros, data for the EF germplasms were transformed by the following formula: $(n + 0.5)$.$^{1/2}$ Because it had no EI version, AU-Triumph was not included in any analysis where infection status was a class variable. It mainly served as an experimental control due to its record of excellent performance in Georgia (Wilkinson et al, 1984). Location effects for yield, stand, and tillering were examined by an overall AOV. Because of a highly significant location effect ($P \leq 0.01$) for each of these variables, separate analyses were conducted for each location.

RESULTS

At Americus, the EF plots of all germplasms had poorer yields during the first year (1988) than their EI counterparts and stands were completely lost by the fall while the EI plots of all germplasms were still very productive at the end of that year (Table 1) and for the duration of the study. AU-Triumph also completely lost its stands at Americus (data not shown). However, no effect of infection status on tillering was seen at any sampling time during 1988 at Americus (data not shown).

Table 3. Seasonal dry matter yield and stands (visual estimation of percent of ground covered with tall fescue during November of each year) of endophyte infected (EI) and endophyte free (EF) versions of four tall fescue germplasms when tested in Tifton, GA for 3 yr.

Year	Germplasm	Status	Yield			Stand
			Winter	Spring	Fall	
			kg ha^{-1}			%
1988	GA-5	EI	1507	6697	2365	98
	GA-5	EF	941	5158	2257	94
	GA-JES	EI	750	5394	3244	99
	GA-JES	EF	752	4284	2311	98
	GA-JESIMP	EI	774	4121	2116	100
	GA-JESIMP	EF	146	5382	2165	98
	GA-ECOKY31	EI	477	5598	2808	99
	GA-ECOKY31	EF	563	4926	2400	96
		LSD (0.05)	422	1103	417	NS
1989	GA-5	ET	1013	810	1198	95
	GA-5	EF	1012	764	891	65
	GA-JES	EI	1077	1024	2396	99
	GA-JES	EF	695,	941		
	GA-JESIMP	EI	819	1007	[illegible]	[illegible]
	GA-JESIMP	EF	850	1031	1374	94
	GA-ECOKY31	EI	1033	1129	2150	98
	GA-ECOKY31	EF	804	917	1380	88
		LSD (0.05)	NS	NS	373	11
1990	GA-5	EI	1321	1242	669	38
	GA-5	EF	1410	1124	436	11
	GA-JES	EI	898	1360	1028	73
	GA-JES	EF	1168	1520	532	33
	GA-JESIMP	EI	892	1375	1565	80
	GA-JESIMP	EF	1003	1607	481	38
	GA-ECOKY31	EI	1667	1415	1182	63
	GA-ECOKY31	EF	1140	1531	773	26
		LSD (0.05)	392	NS	476	21

At Watkinsville, there were no effects of infection status, germplasms, or their interaction over the 3-yr period for yield or percent stand (Table 2). Tillering during the February sampling date of the first year was increased by infection at Watkinsville, but this increase was not found for the remaining sampling dates (data not shown). At Tifton, however, there was a significant ($P \leq 0.05$) year-by-germplasm-by-infection-status interaction with each EF germplasm showing progressively poorer stands than their EI counterparts until the fall of 1990 when all EF plots had significantly ($P \leq 0.05$) less stand (Table 3). Au-Triumph also showed the greatest stand deterioration of all the EF germplasms (data not shown, with <10% stand survival by the fall of 1990). Yields at Tifton generally showed the same trends as stand percentage, but by the fall of 1989, the EI versions of GA-ECOKY31, GA-JES, and GA-JESIMP gave significantly higher yields than their EF counterparts (Table 3). Although more variable in the winter, these yield results carried over into 1990 (Table 3). Also at Tifton, the EI plots showed a significantly higher yield when

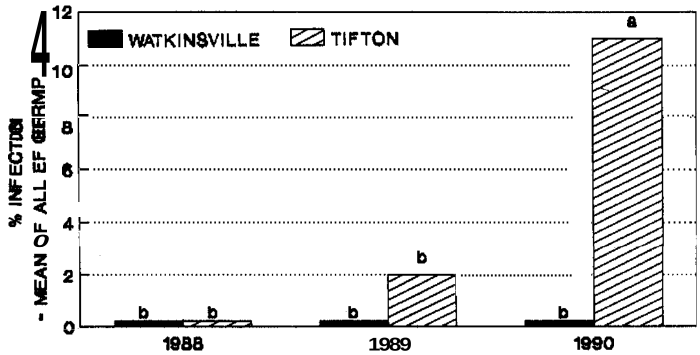

Fig. 1. Percent infection of plots of endophyte-free (EF) tall fescue (data pooled for all germplasms) when grown for three years at Tifton and Watkinsville, GA. Means with the same letter are not significantly different ($p < 0.05$) based on an LSD value calculated on data subjected to the following square root transformation: $(n + 0.5)^{1/2}$.

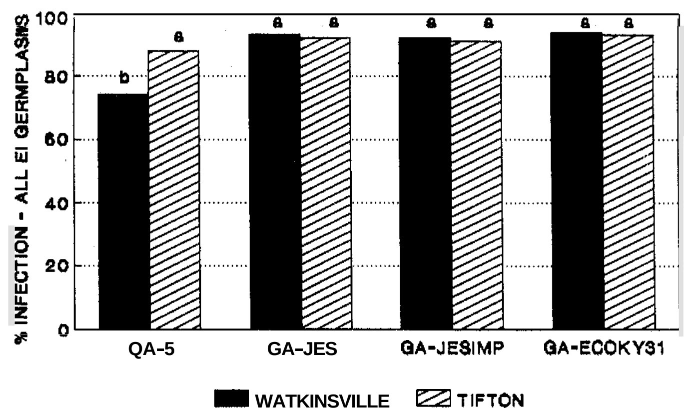

Fig. 2. Percent infection of plots of four endophyte-infected (EI) tall fescue germplasms when grown for 3 yr at Tifton and Watkinsville, GA. Means with the same letter are not significantly different ($p < 0.05$) based on an LSD value calculated from data subjected to an arcsin transformation.

pooled across germplasms during the fall of 1990 (EI = 1074 kg ha^{-1} and EF = 556 kg ha^{-1}), but not in the winter and spring of that same year (data not shown). Similiar to the results found at Watkinsville, tillering was increased because of infection at Tifton only at the February sampling date (data not shown).

Another interesting development was the change in percentage infection of the plots during the course of the study. For the EF germplasms the year-by-location effect was highly significant (P ■ 0.01) and the data were interpreted in light of that interaction. Infection increased significantly (P ■ 0.05) at Tifton for all EF germplasms with the final infection levels ranging from 2% in GA-JES to 41% in GA-5, but no change was found for these same germplasms at Watkinsville (Fig. 1). For the EI plots, the location by germplasm interaction was the most significant effect (P ■ 0.05) with the GA-5 EI plots at Watkinsville showing significantly lower infection levels than the EI plots at Tifton and was the only germplasm to do so (Fig. 2).

DISCUSSION

Generally, EI tall fescue clones were reported to produce more herbage dry matter, tillers, and root growth in the greenhouse (De Battista et al., 1990; Hill et al., 1990) and to be more competitive in the field (Hill et al., 1991) when compared to EF versions of the same clones. Simililarly, when the same germplasms used in this current study were compared in the greenhouse, the influence of infection on yield and tillering was variable (De Battista et al., 1990). However, Bacon and De Battista (1991) found that most studies investigating important agronomic traits associated with infected grasses have not been conducted under environmentally stressful field conditions. This was not the case with our Americus location where the results were startlingly clear; EF swards of all germplasms in all reps completely lost stands within 1 yr. These results, and those from Tifton, strongly indicate that the ecological fitness of EI germplasms is so pronounced that only the use of EI tall fescue can be dependably recommended for the southeastern coastal plain.

Further support of this finding of EI plants being more persistent than EF plant? was also seen with the change in the overall infection level of the plots. Although initially not detectable, infection increased progressively at Tifton for all EF germplasms with GA-5 showing the highest terminal value at 41% (Fig. 1). Infection also increased for the GA-5 EI at Tifton (Fig. 2). These values matched quite well with the stand deterioration of the EF plots during that same time (Table 3). The same trends of essentially EF plots showing progressively more infection in the field were also reported by Read and Walker (1990) in north Texas. This report, plus findings from our study, indicate a higher mortality of EF plants than EI plants which results in greater stand infection levels when percentages are based on the remaining plants as is done with normal pasture sampling. The ecological ramifications for pasture management of these data would be that given enough time and stress in even normal tall fescue growing areas, pastures initially low in endophyte infection could become highly infected. This could then lead to animal toxicoses.

Results at Walkinsville demonstrate that in the current tall fescue growing areas, at least under the less stressful clipping management applied to our plots, EF tall fescue can survive as well as EI plants for short periods. Grazing, however, does impose a unique stress, especially in conjunction with dry weather and will probably lead to greater stand loss of EF plants. Selection for more persistent EF cultivars will probably be necessary to improve the agronomic value of EF tall fescue. Because EF tall fescue allows greater animal performance than EI tall fescue (Studemann and Hoveland, 1988), this is a worthwhile breeding objective.

Since infected tall fescue can cause a variety of toxicities in ruminants (Studemann and Hoveland, 1988), it will be important that management systems be employed

Table 4. Cumulative rainfall totals for the June-August period at three Georgia locations for 3 yr.

Year	Americus	Tifton	Watkinsville
		mm	
Normal*	345.4	388.6	302.3
1988	148.6	265.2	218.4
1989	482.6	413.2	508.0
1990	172.7	222.5	302.3

* Average long-term totals (minimum of 30 yr).

which reduce the detrimental effects of infection if EI tall fescue is to be used to provide economical and reliable forage for wintering cattle in the region. In this regard, growing GA-5 EI tall fescue in mixtures with other pasture species such as bermudagrass *(Cynodon dactylon* L.) or bahiagrass *(Paspalum notatum* Flugge) was found to give acceptable winter gains on yearling beef heifers in the absence of hay and apparently these warm season grasses diluted the effect of the toxic alkaloids during the spring and summer (Gates et al., 1991).

Bacon and De Battista (1991) reported several biotic and abiotic stresses that may individually or collectively be responsible for reducing the persistence of EF tall fescue. Since Arachevaleta et al. (1989) found EI plants to resist drought more than their EF counterparts, we felt drought or lack of rainfall would be the stress most important to monitor at all locations in this study. Also, because stands consistently deteriorated during the summer months in these studies, summer rainfall deviations from the normal were checked at each location and found to be greatest at Americus and least at Tifton and Watkinsville during 1988 (Table 4). This directly matches the stand deterioration at these locations during that year (Tables 1, 2, and 3). When comparing the rainfall deviations for Tifton and Watkinsville, each EF germplasm was found to emerge from the summer of 1989 with good stands which was also a time when rainfall was in surplus at both locations (Table 4). However, stands thinned badly at Tifton after the summer of 1990 (Table 3) which also corresponded with a severe rainfall deficit during that same year at Tifton which was not seen at Watkinsville (Table 4). Therefore, although other stress factors such as pests or competition from warm season grasses were probably contributors to the stand deterioration of our EF plots, it is felt that summer drought played the major role in this deterioration.

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